FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 217677US0PCT

10/019284

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): 09/926853

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP00/04348	30 June 2000	2 July 1999

TITLE OF INVENTION
DNA ENCODING SUCROSE PTS ENZYME II

APPLICANT(S) FOR DO/EO/US
IZUI Masako et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

PCT/IB/304/Drawings (2 sheets)
PCT/IB/308/Notice of Priority
Form PTO-1449/Form PTO-1595
Sequence Listing(14 pages)

531 Rec'd PCT/PTO 02 JAN 2002

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/070192	PCT/JP00/04348	217677US0PCT

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1040.00**
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	**$890.00**
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$740.00**
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$710.00**
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**

ENTER APPROPRIATE BASIC FEE AMOUNT = **$890.00**

Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)). **$0.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	3 - 20 =	0	x $18.00	**$0.00**
Independent claims	2 - 3 =	0	x $84.00	**$0.00**
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**

TOTAL OF ABOVE CALCULATIONS = **$890.00**

☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2. **$0.00**

SUBTOTAL = **$890.00**

Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). + **$0.00**

TOTAL NATIONAL FEE = **$890.00**

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☒ **$40.00**

TOTAL FEES ENCLOSED = **$930.00**

Amount to be: refunded $

charged $

a. ☒ A check in the amount of **$930.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **15-0030** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Surinder Sachar
Registration No. 34,423

22850

Surinder Sachar
SIGNATURE

Norman F. Oblon
NAME

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REGISTRATION NUMBER

Jan 2 2002
DATE

217677USPCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :

Masaka IZUI, et al. :

SERIAL NO: 10/019,284 : ATTN: BOX SEQUENCE

FILED: January 2, 2002 :

FOR: DNA ENCODING SUCROSE PTS ENZYME II

PRELIMINARY AMENDMENT AND STATEMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

In response to the Notice of March 11, 2002, Applicants respectfully submit herewith an amendment, Substitute Sequence Listing, and a computer-related Sequence Listing.

IN THE SPECIFICATION

At page 29, after the last line, on the next page, please delete the original Sequence Listing from pages 1-14 and insert the Substitute Sequence Listing attached hereto.

REMARKS

Claims 1-3 are pending.

Contents of the paper copy of the Substitute Sequence Listing and the computer-readable Sequence Listing filed herewith are identical. Support for all the sequences listed in the Substitute Sequence Listing can be found in the present application. No new matter is

believed to be introduced by the submission of the Substitute Sequence Listing and the computer-readable Sequence Listing.

Applicants submit that this application is in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
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Marked-Up Copy
Serial No: 10/019,284
Amendment Filed on:
HEREWITH

IN THE SPECIFICATION

At page 29, after the last line, on the next page, please delete the original Sequence Listing from pages 1-14 and insert the Substitute Sequence Listing attached hereto.

#13

217677US0PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :

Masaka IZUI, et al. :

SERIAL NO: 10/019,284 : ATTN: BOX SEQUENCE

FILED: January 2, 2002 :

FOR: DNA ENCODING SUCROSE PTS ENZYME II

PRELIMINARY AMENDMENT AND STATEMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

In response to the Communication apparently dated September 24, 2002, Applicants respectfully submit herewith an amendment, Substitute Sequence Listing, and a substitute computer-readable Sequence Listing.

IN THE SPECIFICATION

At page 29, after the last line, on the next page, please delete the Sequence Listing filed May 13, 2002, and insert the Substitute Sequence Listing attached hereto.

REMARKS

Claims 1-3 are pending.

Applicants received the attached Communication from the USPTO with a fully torn cover sheet. Therefore, Applicants were unaware of the due date for replying to the Communication. Thereafter, Applicants attempted to contact Mark Spencer at the USPTO to

clarify the matter. Mark Spencer did not return our phone call until October 28,2002. During this telephone conversation, Mark Spencer indicated that Applicants must contact OIPE at the USPTO to clarify any communication from the USPTO. Thereafter, Applicants contacted the general information desk at OIPE which indicated that the information about the Communication was unavailable. Applicants then contacted Ms. Erin Penders at the PCT department at the USPTO, who indicated that she has no record of the attached communication being mailed to Applicants. However, Ms. Penders indicated that she would retrieve the case and advise Applicants therefrom. On December 5, 2002, Applicants contacted Ms. Penders again regarding the matter. Although Ms. Penders indicated that she did not retrieve the case as of that date, she did indicate that the Communication may have been mailed September 24, 2002. To this date, Applicants have not been contacted by Ms. Penders while in possession of the case to establish the proper due date for the enclosed response. Therefore, Applicants enclose herewith, a substitute Sequence Listing and substitute computer readable Sequence Listing in response.

Contents of the paper copy of the Substitute Sequence Listing and the computer-readable Sequence Listing filed herewith are identical. Support for all the sequences listed in the Substitute Sequence Listing can be found in the present application. No new matter is believed to be introduced by the submission of the Substitute Sequence Listing and the computer-readable Sequence Listing.

Applicants submit that this application is in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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217677US0PCT

Marked-Up Copy
Serial No: 10/019,284
Amendment Filed on:
HEREWITH

IN THE SPECIFICATION

--At page 29, after the last line, on the next page, please delete the Sequence Listing filed May 13, 2002, and insert the Substitute Sequence Listing attached hereto.--

SPECIFICATION

DNA Encoding Sucrose PTS Enzyme II

Technical Field

The present invention relates to a DNA encoding sucrose PTS enzyme II, which is a protein involved in uptake of sucrose into a cell of coryneform bacterium.

Background Art

Bacteria can assimilate many carbon sources, and various specific systems exist for their cellular transmembrane transport. Moreover, most of bacteria can respond to environmental changes to survive under a limited nutritious condition. Their cells are provided with a detector for monitoring the environment to select their nutrition from various carbon sources. Examples of such transmembrane transport systems and detectors of sugars include PTS (phosphoenolpyruvate/carbohydrate phosphotransferase system or phosphoenolpyruvate-sugar transport system; as for PTS, refer to *Escherichia coli* and *Salmonella* Cellular and Molecular Biology, Second Edition, ASM (American Society for Microbiology) Press).

PTS is involved in regulation of transmembrane transport and phosphorylation of various sugars (PTS sugars), movement towards these carbon sources and many metabolic pathways. PTS catalyzes the following

reaction. PEP refers to phosphoenolpyruvic acid.

PEP (intracellular) + Sugar (extracellular) -->
Pyruvic acid (intracellular) +
Phosphorylated sugar (intracellular)

PTS catalyzes a reaction for generating a phosphorylated sugar and pyruvic acid by translocating a phosphate group of intracellular phosphoenolpyruvic acid (also referred to as "PEP" hereafter) to an extracellular sugar. The phosphorylation of a sugar is linked with cellular transmembrane transport of a sugar, and energies required for these processes are supplied from PEP, which is an intermediate of the glycolytic pathway.

In *Escherichia coli* and *Salmonella typhimurium*, proteins constituting PTS catalyze the following reactions.

(1) PEP + EI --> P-EI + Pyruvic acid

(2) P-EI + Hpr --> P-Hpr + EI

(3) P-Hpr + EIIA --> P-EIIA + Hpr

(4) P-EIIA + EIIB --> P-EIIB + EIIA

(5) P-EIIB + Sugar (extracellular) + EIIB + Sugar-P (intracellular)

Among proteins involved in the above reactions, EI (Enzyme I) and Hpr (histidine protein) are soluble cytoplasmic proteins involved in phosphorylation of all

PTS sugars and referred to as general PTS proteins.

On the other hand, EII (Enzyme II) is specific for PTS sugars and consists of several domains or proteins depending on the sugars. For example, the mannitol-specific EII is a membrane-bound protein consisting of three domains, A, B and C. The glucose-specific EII and sucrose-specific EII consist of IIB and IIC, which are membrane-bound proteins, and IIA, which is a soluble protein. In any case, translocation of a phosphate group from PEP to a sugar is mediated by EI, HPr, EIIA and EIIB. The EIIC domain, which is an intramembranous portion of EII, forms a translocation channel and is considered to be probably a specific binding site of a substrate.

The third type of EII is observed in mannose PTS. Both of its domains A and B are fused in a single soluble polypeptide, and the two intramembranous proteins (IIC and IID) are involved in transmembrane transport of mannose.

In *Escherichia coli* and *Salmonella typhimurium*, the gene encoding EI (ptsI) has been cloned and sequenced (Saffen, E.W. *et al.*, *J. Biol. Chem.*, *262*, pp.16241-16253, 1987; De Reuse, H. and Danchin, A., *J. Bacteriol.*, *170*, pp.3827-3837, 1988). Further, EII specific for some sugars have also been cloned (Saffen, E.W. *et al.*, *J. Biol. Chem.*, *262*, pp.16241-16253, 1987; Erni, B. and Zanolari, B., *J. Biol. Chem.*, *261*,

pp.16398-16403, 1986; Nelson, S.O. *et al.*, *EMBO J.*, *3*, pp.1587-1593, 1984).

It is known that some kinds of sugars are taken up by non-PTS, which do not require PEP, as a system for uptake into cells.

Disclosure of the Invention

As described above, many studies about uptake of sugar into cells have been performed, but studies about PTS in industrially useful coryneform bacteria have not made much progress. Accordingly, an object of the present invention is to provide a gene encoding a protein constituting sucrose PTS in coryneform bacterium.

The inventors of the present application isolated a DNA fragment including a gene encoding sucrase (invertase) of coryneform bacterium and determined its structure. Further, they developed a method for producing an amino acid or a nucleic acid by using a coryneform bacterium containing the amplified sucrase gene (Japanese Patent Laid-open Publication (Kokai) Nos. 5-244958 and 8-196280). In the DNA fragment, four open reading frames (ORF-F1, ORF-F2, ORF-F3 and ORF-F4) exist in a *Sma*I fragment of about 6 kb.

However, the inventors of the present invention considered based on comparison with other sucrase genes that the aforementioned ORF-F2 did not contain the sucrase gene in full length. That is, the number of

amino acid residues in sucrase estimated from known sucrase genes is 466 to 511 (Gunaseakren, P., *J. Bacteriol., 172* (12), pp.6727-35, 1990), whereas the amino acid sequence that can be encoded by ORF-F2 contains 424 amino acid residues, which was relatively short. Therefore, a sequence existing downstream from ORF-F2 was cloned again and its nucleotide sequence was determined. As a result, it was revealed that the DNA fragment containing the aforementioned sucrase gene consisted of two independent cloned fragments ligated to each other, and it was found that a novel gene encoding sucrose PTS enzyme II existed downstream from the sucrase gene. Thus, the present invention was accomplished.

That is, the present invention provides a protein defined in the following (A) or (B):

(A) a protein which has the amino acid sequence of SEQ ID NO: 2 in Sequence Listing;

(B) a protein which has the amino acid sequence of SEQ ID NO: 2 in Sequence Listing including substitution, deletion, insertion, addition or inversion of one or several amino acids, and an activity for binding to sucrose.

The present invention also provides a DNA which encodes a protein defined in the following (A) or (B):

(A) a protein which has the amino acid sequence of SEQ ID NO: 2 in Sequence Listing;

(B) a protein which has the amino acid sequence of SEQ ID NO: 2 in Sequence Listing including substitution, deletion, insertion, addition or inversion of one or several amino acids, and an activity for binding to sucrose.

The aforementioned DNA includes a DNA defined in the following (a) or (b):

(a) a DNA which contains the nucleotide sequence of the nucleotides 3779 to 5761 of SEQ ID NO: 1 in Sequence Listing;

(b) a DNA which is hybridizable with a nucleotide sequence containing the nucleotide sequence of the nucleotides 3779 to 5761 of SEQ ID NO: 1 in Sequence Listing under a stringent condition, and encodes a protein having an activity for binding to sucrose.

Brief Description of the Drawings

Fig. 1 shows a construction process of a plasmid for disrupting the sucrose PTS enzyme II gene.

Fig. 2 shows a construction process of pBCT4.

Best Mode for Carrying out the Invention

Hereafter, the present invention will be explained in detail.

The DNA of the present invention was obtained, in the examples described later, by amplifying a region existing downstream from the sucrase gene on chromosomal

DNA of *Brevibacterium lactofermentum* by PCR (polymerase chain reaction).

A region adjacent to a known region on a chromosomal DNA can be amplified by ligating a cassette to a DNA fragment containing the regions and performing PCR using a primer corresponding to the known region and a primer corresponding to the cassette. At this time, if the 5' end of the cassette is dephosphorylated beforehand, a nick is generated at a ligation site of the chromosomal DNA fragment and 5' end of the cassette. Therefore, DNA synthesis started from the cassette primer will be stopped at this ligation site, and only the DNA synthesized from a synthetic primer will serves as a template for synthesis starting from the cassette primer and a complementary chain will be formed. As a result, specific amplification becomes possible (cassette-ligation mediated PCR method (*Molecular and Cellular Probes*, *6*, pp.467-475)). A kit utilizing this method is commercially available (TAKARA LA PCR™ in vitro Cloning Kit, Takara Shuzo) and can be utilized to obtain the DNA of the present invention.

Since the nucleotide sequences of the DNA of the present invention and the adjacent region thereof have been revealed, they can be directly amplified by PCR using oligonucleotides synthesized based on these nucleotide sequences as primers and chromosomal DNA of coryneform bacterium as a template. Examples of such

primers include oligonucleotides having the nucleotide sequences of SEQ ID NOS: 10 and 21. Further, the DNA of the present invention can also be isolated from a chromosomal DNA library by hybridization using an oligonucleotide synthesized based on these nucleotide sequences as a probe. The chromosomal DNA of coryneform bacterium can be obtained by, for example, the method of Saito *et al.* (described in *Biochim. Biophys. Acta, 72,* pp.619-629, 1963) or the method of K. S. Kirby (*Biochem. J., 64,* p.405, 1956).

Further, conventional methods well known to those skilled in the art can be employed for preparation of chromosomal DNA, preparation of chromosomal DNA library, hybridization, PCR, preparation of plasmid DNA, digestion and ligation of DNA, transformation, design of oligonucleotides used as primers and so forth. These methods are described in Sambrook, J., Fritsch, E. F., and Maniatis, T., "Molecular Cloning A Laboratory Manual, Second Edition", Cold Spring Harbor Laboratory Press, 1989 and so forth.

Plasmids used for cloning of the DNA of the present invention, preparation of a chromosomal DNA library or the like may be those that can replicate in microorganisms such as bacteria belonging to the genus *Escherichia*, and specific examples thereof include pBR322, pTWV228, pMW119, pUC19 and so forth.

An example of the nucleotide sequence of a DNA

fragment containing the DNA of the present invention obtained as described above is shown as SEQ ID NO: 1 in Sequence Listing. The region containing the nucleotide sequence of the nucleotides 3779 to 5761 in this nucleotide sequence encodes sucrose PTS enzyme II, which is the protein of the present invention. In the nucleotide sequence of SEQ ID NO: 1, the nucleotides 342 to 1505 and the nucleotides 2338 to 3609 correspond to ORF-F1 and ORF-F2, respectively, in the DNA fragment containing the sucrase gene described in Japanese Patent Laid-open Publication (Kokai) No. 8-196280. Further, when the nucleotide sequence of SEQ ID NO: 1 and the nucleotide sequence described in Japanese Patent Laid-open Publication (Kokai) No. 8-196280 are compared, a region of the nucleotides 1 to 3687 in the nucleotide sequence of SEQ ID NO: 1 was identical to the nucleotide sequence described in Japanese Patent Laid-open Publication (Kokai) No. 8-196280. Accordingly, it was revealed that the DNA fragment containing the sucrase gene consisted of two independent cloned fragments.

The DNA of the present invention may be a DNA that encodes sucrose PTS enzyme II including substitution, deletion, insertion, addition or inversion of one or several amino acids at one or a plurality of sites so long as the activity for binding to sucrose of the encoded sucrose PTS enzyme II is not deteriorated. The number meant by the term "several" used herein may vary

depending on locations of amino acid residues in the three-dimensional structure of proteins and kinds of amino acid residues. This is due to the fact that there are highly analogous amino acids among amino acids such as isoleucine and valine, and difference among such amino acids does not substantially affect the three-dimensional structure of proteins. Therefore, the protein may be one having homology of 70% to 80% or higher, preferably, 90% to 95%, with respect to the whole amino acid sequence constituting sucrose PTS enzyme II and having an activity for binding to sucrose. Specifically, the term "several" means 2 to 180, preferably 2 to 60, more preferably 2 to 5.

Such a DNA encoding a protein which is substantially identical to sucrose PTS enzyme II as mentioned above can be obtained by modifying a nucleotide sequence so that the amino acid sequence at a particular site should include substitution, deletion, insertion, addition or inversion of an amino acid residue or residues through, for example, site-specific mutagenesis. Further, such a modified DNA as mentioned above may also be obtained by a conventional mutagenesis treatment. Examples of the mutagenesis treatment include an in vitro treatment of DNA encoding sucrose PTS enzyme II with hydroxylamine or the like, a treatment of microorganisms such as *Escherichia* bacteria containing the DNA encoding sucrose PTS enzyme II by UV

irradiation or with mutagenesis agents used for a usual mutagenesis treatment such as N-methyl-N'-nitro-N-nitrosoguanidine (NTG) and nitrous acid.

The aforementioned substitution, deletion, insertion, addition, inversion or the like of nucleotides includes naturally occurring mutations (mutant or variant) such as those observed depending on differences of strains, species or genera of microorganisms containing sucrose PTS enzyme II and so forth.

A DNA which encodes a protein substantially the same as sucrose PTS enzyme II can be obtained by, for example, isolating a DNA which is hybridizable with a DNA having the nucleotide sequence of the nucleotides 3779 to 5761 of the nucleotide sequence of SEQ ID NO: 1 in Sequence Listing or a probe prepared from DNA having the nucleotide sequence by PCR or the like under stringent conditions and encoding a protein containing sucrose PTS enzyme II having an activity for binding to sucrose from DNA encoding sucrose PTS enzyme II containing a mutation or a cell containing it. The "stringent conditions" referred to herein is a condition under which a so-called specific hybrid is formed, but a non-specific hybrid is not formed. It is difficult to clearly define this condition by using numerical values. However, for example, the stringent conditions include a condition under which two of DNAs having high homology,

for example, two of DNAs having homology of not less than 50% are hybridized with each other, but two of DNAs having homology lower than the above level are not hybridized with each other. Alternatively, the stringent conditions are exemplified by a hybridization condition represented by salt concentrations of 1 x SSC, 0.1% SDS, preferably 0.1 x SSC, 0.1% SDS, at 60°C, which is an ordinary condition of washing in Southern hybridization. The homology used herein is represented with a value calculated by the method of Lipman-Pearson (*Science*, *227*, pp.1435-1441, 1985) or the method of Takashi & Gotoh (*J. Biochem.*, *92*, pp.1173-1177, 1984). The probe can be designed according to a method known to those skilled in the art.

Those genes hybridizable under the condition as described above include those having a stop codon generated in the genes, but such genes can be easily removed by ligating them to a commercially available expression vector to examine size of the expressed product.

The protein of the present invention is a protein encoded by the DNA of the present invention and has the amino acid sequence of SEQ ID NO: 2. The protein of the present invention may have an amino acid sequence corresponding to the amino acid sequence of SEQ ID NO: 2 in Sequence Listing including substitution, deletion, insertion, addition or inversion of one or several amino

acids so long as it has an activity for binding to sucrose.

The DNA of the present invention can be utilized to improve sucrose uptake ability of coryneform bacteria or the like. Further, since PTS consumes PEP for uptake of a sugar into a cell, PTS is considered to be disadvantageous for synthesis of amino acids of which biosynthesis system include PEP in an upstream stage. Therefore, if sucrose PTS is disrupted and sucrose can be taken up by an uptake system which does not require PEP, it is considered advantageous in view of sucrose uptake rate or productivity of an amino acid or the like. In coryneform bacteria, non-PTS specific for sucrose is not known, but, for example, if sucrase is allowed to act extracellularly, glucose and fructose can be taken up by non-PTS.

Further, if the DNA of the present invention is modified so as to encode sucrose PTS enzyme II having an enhanced or suppressed function or so as to be ligated to an expression control sequence such as a promoter derived from other genes and introduced into a coryneform bacterium, a coryneform bacterium having an enhanced or suppressed sucrose uptake ability can be created. Specifically, a DNA encoding sucrose PTS enzyme II having an enhanced function is introduced into an autonomously replicable vector or chromosomal DNA in a cell of coryneform bacterium. Further, a DNA encoding

sucrose PTS enzyme II having a suppressed function is introduced into chromosomal DNA by gene substitution utilizing homologous recombination. Alternatively, a coryneform bacterium in which sucrose PTS functions at low temperature but does not function at high temperature can be created by gene substitution using a plasmid containing a temperature sensitive replication control region (see Japanese Patent Publication (Kokoku) No. 7-108228).

Coryneform bacteria to which the present invention is applicable include those bacteria having been hitherto classified into the genus *Brevibacterium* but united into the genus *Corynebacterium* at present (*Int. J. Syst. Bacteriol., 41*, 255 (1981)), and include bacteria belonging to the genus *Brevibacterium* closely relative to the genus *Corynebacterium*. Examples of such coryneform bacteria are mentioned below.

Corynebacterium acetoacidophilum

Corynebacterium acetoglutamicum

Corynebacterium alkanolyticum

Corynebacterium callunae

Corynebacterium glutamicum

Corynebacterium lilium (*Corynebacterium glutamicum*)

Corynebacterium melassecola

Corynebacterium thermoaminogenes

Corynebacterium herculis

Brevibacterium divaricatum (*Corynebacterium glutamicum*)

Brevibacterium flavum (*Corynebacterium glutamicum*)

Brevibacterium immariophilum

Brevibacterium lactofermentum (*Corynebacterium glutamicum*)

Brevibacterium roseum

Brevibacterium saccharolyticum

Brevibacterium thiogenitalis

Brevibacterium ammoniagenes (*Corynebacterium ammoniagenes*)

Brevibacterium album

Brevibacterium cerium

Microbacterium ammoniaphilum

Examples of the vector autonomously replicable in a cell of coryneform bacterium include pAM330 (refer to Japanese Patent Laid-open (Kokai) No. 58-67699), pHM1519 (refer to Japanese Patent Laid-open (Kokai) No. 58-77895) and so forth. Moreover, if a DNA fragment having an ability to make a plasmid autonomously replicable in coryneform bacterium is excised from these vectors and inserted into the vectors for *Escherichia coli*, they can be used as a so-called shuttle vector autonomously replicable in both of *Escherichia coli* and coryneform bacteria. Examples of such a shuttle vector include those mentioned below. There are also indicated microorganisms that harbor each vector, and accession

numbers thereof at international depositories are shown in the parentheses, respectively. Among these, pHSC4 includes a temperature sensitive replication control region.

pAJ655	*Escherichia coli* AJ11882 (FERM BP-136)	
	Corynebacterium glutamicum SR8201 (ATCC39135)	
pAJ1844	*Escherichia coli* AJ11883 (FERM BP-137)	
	Corynebacterium glutamicum SR8202 (ATCC39136)	
pAJ611	*Escherichia coli* AJ11884 (FERM BP-138)	
pAJ3148	*Corynebacterium glutamicum* SR8203 (ATCC39137)	
pAJ440	*Bacillus subtilis* AJ11901 (FERM BP-140)	
pHC4	*Escherichia coli* AJ12617 (FERM BP-3532)	
pHSC4	*Escherichia coli* AJ12571 (FERM BP-3524)	

A recombinant vector containing the DNA of the present invention can be introduced into a coryneform bacterium according to a transformation method reported so far. For instance, there are a method of treating recipient cells with calcium chloride so as to increase the permeability of DNA, which has been reported for *Escherichia coli* K-12 (Mandel, M. and Higa, A., *J. Mol. Biol.*, *53*, 159, 1970); and a method of preparing competent cells from cells which are at the growth phase followed by introducing the DNA thereinto, which has been reported for *Bacillus subtilis* (Duncan, C.H., Wilson, G.A. and Young, F.E., *Gene*, *1*, 153, 1977). In

addition to these, also employable are a method of making DNA-recipient cells into protoplasts or spheroplasts, which can easily take up recombinant DNA, followed by introducing the recombinant DNA into the DNA-recipient cells, which method is known to be applicable to *Bacillus subtilis*, actinomycetes and yeasts (Chang, S. and Choen, S.N., *Molec. Gen. Genet., 168*, 111, 1979; Bibb, M.J., Ward, J.M. and Hopwood, O.A., *Nature, 274*, 398, 1978; Hinnen, A., Hicks, J.B. and Fink, G.R., *Proc. Natl. Acad. Sci., USA, 75*, 1929, 1978) and the electric pulse method (see Japanese Patent Laid-open Publication (Kokai) No. 2-207791).

Examples

Hereafter, examples of the present invention will be explained in detail.

Example 1: Isolation of gene encoding sucrose PTS enzyme II

<1> Analysis of chromosomal DNA of *Brevibacterium lactofermentum* AJ12036 (FERM BP-734) by Southern hybridization

The *Brevibacterium lactofermentum* AJ12036 strain was cultured overnight in 4 ml of M-CM2S medium (containing 5 g/L of sucrose, 10 g/L of polypeptone, 10 g/L of yeast extract, 5 g/L of NaCl and 0.1 g/L of DL-methionine) and microbial cells were collected.

Chromosomal DNA was extracted from the obtained microbial cells by using a Bacterial Geneomic DNA Purification Kit (Advanced Genetic Technologies). The chromosomal DNA was eluted with 50 μl of TE buffer (composition: 10 mM tris-HCl (pH 7.5), 1 mM EDTA-2Na).

The chromosomal DNA extracted as described above was subjected to Southern hybridization according to the method described in Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, 1989. The chromosomal DNA was separately digested with *Bam*HI and *Sma*I, which did not cleave regions on the C-terminus side of ORF-F2 and N-terminus side of ORF-F3 and subjected to agarose gel electrophoresis. As a probe, a fragment of about 3 kb was used that was excised from 6.9 kb fragment cloned on pSSM30 (Japanese Patent Laid-open Publication (Kokai) No. 8-196280) with *Bam*HI to cover the regions on the C-terminus side of ORF-F2 and on the N-terminus side of ORF-F3 (Japanese Patent Laid-open Publication (Kokai) No. 8-196280, the fragment of SEQ ID NO: 1649 to 4675 in Sequence Listing).

As a result of the hybridization, two bands were detected, and it was revealed that ORF-F2 and ORF-F3 were not adjacent to each other on the chromosome. Therefore, it was attempted to confirm a sequence existing downstream from the sucrase gene again.

<2> Determination of sequence of region existing downstream from sucrase gene

To determine the nucleotide sequence of a region downstream from the sucrase gene, the downstream region was first amplified by PCR. PCR was performed by using a TAKARA LA PCR™ in vitro Cloning Kit (Takara Shuzo). Specifically, the PCR was performed as follows.

The chromosomal DNA was completely digested with 10 kinds of restriction enzymes (*Spe*I, *Eco*T14I, *Nhe*I, *Pst*I, *Eco*T22I, *Bgl*II, *Bam*HI, *Xho*I, *Sal*I, *Ava*I), which produced the same cleavage ends as cassettes (SEQ ID NOS: 3 to 8 in Sequence Listing) attached to the aforementioned kit. PCR was performed by using each of these fragments as a template, Synthetic primer 1 shown in Table 1 and Cassette primer 1 (SEQ ID NO: 19). Since a phosphate group was not added to the 5' end of the cassette, a nick was generated at the ligation site of the chromosomal DNA fragment and the 5' end of the cassette. Therefore, the DNA synthesis starting from the cassette primer stopped at this ligation site, and only the DNA synthesized from the synthetic primer served as a template for synthesis starting from the cassette primer, and a complementary chain was formed.

Subsequently, PCR was performed out by using the amplification product obtained above as a template, Synthetic primer 2 and Cassette primer 2 (SEQ ID NO: 20). As a result, a fragment could be amplified when a DNA

obtained by digesting the chromosomal DNA with *Eco*T14I, *Pst*I, *Bgl*II, *Bam*HI, *Xho*I or *Ava*I was used as a template. The nucleotide sequence of a fragment of about 1.8 kb amplified by using the DNA fragment digested with *Bam*HI as a template was determined.

Table 1: Nucleotide sequence and position of synthetic primer

Primer number	Nucleotide sequence	Location in SEQ ID NO: 1 (nucleotide number)
1	CGTCTTGCGAGGATTCAGCGAGCTG (SEQ ID NO: 9)	(3159 to 3183)
2	AGCTGGATTTCGGCCATGAATTCTA (SEQ ID NO: 10)	(3179 to 3203)
3	GATCTGTTCGGTCCGCAATCACT (SEQ ID NO: 11)	(4189 to 4212)
4	CACTGGTGGAGATGTTCCCTCAGAT (SEQ ID NO: 12)	(4209 to 4233)
5	CATCTTCGCAACCGCATCCATGGCC (SEQ ID NO: 13)	(4801 to 4825)
6	CGCGCAGGGTGCAGCATGTTTGGC (SEQ ID NO: 14)	(4831 to 4854)
7	GGGCCTTGCAGGTGCTTCAGGTGTC (SEQ ID NO: 15)	(4888 to 4912)
8	CCGCTGTTCTTGGTATTACAGAGCC (SEQ ID NO: 16)	(4914 to 4938)
9	GCAGCGTCAGCGATGCCATGTTTGC (SEQ ID NO: 17)	(5322 to 5346)
10	GCTTGGCTCAGGTGTTGCGATCGTC (SEQ ID NO: 18)	(5356 to 5380)

Synthetic primers 3 and 4 were synthesized based on the determined sequences. In the same manner as described above, the fragments were successively amplified by PCR using a combination of Synthetic primer 3 and Cassette primer 1 and a combination of Synthetic

primer 4 and Cassette primer 2. As a result, a fragment could be amplified when a DNA obtained by digesting the chromosomal DNA with *Pst*I or *Bam*HI was used as a template. The nucleotide sequence of the fragment amplified based on the DNA fragment digested with *Pst*I was determined.

Synthetic primers 5 and 6 were synthesized based on the determined sequence. PCR was successively carried out by using a combination of Synthetic primer 5 and Cassette primer 1 and a combination of Synthetic primer 6 and Cassette primer 2. As a result, an amplified fragment could be confirmed when the chromosomal DNA digested with *Eco*T14 or *Pst*I was used as a template. The nucleotide sequence of the fragment of the former case was determined.

Further, Synthetic primers 7 and 8 were synthesized and the same procedure as described above was performed. As a result, an amplified fragment could be confirmed when *Eco*T14-digested chromosomal DNA was used as a template. The nucleotide sequence of this amplified fragment was determined.

Primers 9 and 10 were synthesized based on the above sequence, and the same procedure as described above was performed. As a result, an amplified fragment could be confirmed when *Spe*I-digested chromosomal DNA was used as a template. The nucleotide sequence of this amplified fragment was determined.

As for the nucleotide sequence determination, a reaction was performed by using a sequencing kit produced by ABI according to its protocol, and then the nucleotide sequence of the amplified fragment was determined by the fluorescence labeling method.

The above results are shown in SEQ ID NO: 1 in Sequence Listing. It was found that a novel ORF existed after the nucleotide number 3684 in this nucleotide sequence. It was inferred that this ORF consisted of the nucleotide sequence of 1983 bp corresponding to the nucleotide numbers 3779 to 5761, and that a protein obtained by translating the determined nucleotide sequence consisted of 661 amino acids. As for the ORF, homology search was performed in the GENBANK CDS database. As a result, as shown in Table 2, the proteins that could be encoded by the ORF showed high homology with sucrose PTS enzyme II, a protein specific for sucrose uptake. Hereafter, this ORF is referred to as ptsIIsuc gene.

Table 2: Results of homology search of novel ORF

Name of Bacterium and gene		Known protein showing homology	Homology (%)
P. pentsaceus	scrA	Enzyme IIscr	48.8
B. subtilis	treP	Trehalose-specific enzyme IIBC	43.4
S. xylosus	scrA	Enzyme IIscr	52.2
S. mutans	scrA	Enzyme IIscr	45.4
S. typhimurium plasmid pUR400	scrA	Enzyme Iiscr	37.6

Example 2: Preparation of sucrose PTS enzyme II gene-disrupted strain

A *Brevibacterium lactofermentum* strain with a disrupted ptsIIsuc gene was prepared. First of all, a plasmid for disrupting the gene was prepared (Fig. 1). A ptsIIsuc gene fragment amplified by PCR using the chromosome of *Brevibacterium lactofermentum* AJ12036 as a template, Primer 2 (SEQ ID NO: 10) and Primer 11 (SEQ ID NO: 21) having the nucleotide sequence shown below was cloned by using a TA cloning kit (Invitrogen), and the plasmid was designated as pCRS2.

(Primer 11)
CGCTACTGCTGAACGAACATGTCC (corresponding to the nucleotide numbers 5947 to 5924 in SEQ ID NO: 1)

A fragment excised from pCRS2 by digestion with *Xba*I and *Spe*I was ligated to the *Xba*I ends of pHSG399 to construct p399S2. This plasmid was digested with *Hpa*I and *Bam*HI, and the obtained fragment (corresponding to

the nucleotide numbers 4385 to 4798 in SEQ ID NO: 1) was ligated to pHSG299 digested with *Sma*I and *Bam*HI to prepare a plasmid pdSB. Subsequently, pdSB was digested with *Bam*HI and ligated to a temperature sensitive replication origin that was excised from plasmid pBCT4 by digestion with *Bam*HI and could replicate in coryneform bacteria (refer to Japanese Patent Publication (Kokoku) No. 7-108228) to prepare a plasmid pdSBT. The plasmid included the ptsIIsuc gene having deleted 5'end and 3' ends. The pdSBT could autonomously replicate in coryneform bacteria at about 10° C to 32° C, but not at about 34° C or higher.

The pBCT4 was constructed as follows. A temperature sensitive vector pHSC4 described in Japanese Patent Publication (Kokoku) No. 7-108228 was digested with restriction enzymes, *Bam*HI and *Kpn*I, to obtain a DNA fragment of about 3 kb containing the obtained temperature sensitive replication origin. Both of the ends of the obtained DNA fragment were blunt-ended with T4 DNA polymerase. This DNA fragment was ligated with *Bam*HI linkers and digested with *Bam*HI again. Then, it was ligated to pHSG399 digested with *Bam*HI to obtain pBCT4 (Fig. 2).

The *Brevibacterium lactofermentum* AJ12036 strain was transformed with pdSBT and a transformant was selected by using a CM2S plate containing 25 μg/ml of kanamycin. The transformation was performed by the

electric pulse method (refer to Japanese Patent Laid-open Publication (Kokai) No. 2-207791). The obtained transformant was designated as AJ12036/pTSBT. The AJ12036/pTSBT strain was diluted and spread on M-CM2S plates containing 25 μg/ml of kanamycin at 10^3 to 10^5 cfu per plate. The transformants on the plates were cultured overnight at 34°C, and a strain showing drug resistance was obtained as a strain containing the plasmid incorporated into its chromosome. It was confirmed by PCR that the obtained strain had the vector plasmid incorporated into the pTSIIsuc gene of the host chromosome by homologous recombination. This integrated strain was designated as YdS1.

The YdS1 strain was cultured at 34°C in a minimal medium containing glucose or sucrose as a sugar source (20g/L of glucose or sucrose, 5 g/L of ammonium sulfate, 2 g/L of urea, 1 g/L of KH_2PO_4, 0.5 g/L of $MgSO_4 \cdot 7H_2O$, 0.002 g/dl of $FeSO_4$, 0.002 g/dl of $MnSO_4$, 100 μg/L of biotin, 2000 μg/L of vitamin B1, 10 mg/dl of DL-methionine and 15 g/L of agar, pH 6.6). The results are shown in Table 3. Since the YdS1 strain could grow in the minimal medium containing only glucose as a carbon source, but not in the minimal medium containing only sucrose as a carbon source, it was confirmed that the ptsIIsuc gene is the gene encoding Enzyme II which is a protein specific for sucrose in sucrose uptake.

Table 3: Growth on minimal medium

Bacterial strain	Carbon source	
	Sucrose	Glucose
AJ12036	Possible to grow	Possible to grow
YdS1	Impossible to grow	Possible to grow

Industrial Applicability

The present invention provides a gene encoding sucrose PTS enzyme II of coryneform bacterium and a strain of coryneform bacterium in which sucrose PTS does not function. These gene and bacterial strain can be utilized in breeding of strains with improved sugar uptake rate or improved productivity of an amino acid, a nucleic acid or the like.

What is claimed is:

1. A protein defined in the following (A) or (B):

(A) a protein which has the amino acid sequence of SEQ ID NO: 2 in Sequence Listing;

(B) a protein which has the amino acid sequence of SEQ ID NO: 2 in Sequence Listing including substitution, deletion, insertion, addition or inversion of one or several amino acids, and an activity for binding to sucrose.

2. A DNA which encodes a protein defined in the following (A) or (B):

(A) a protein which has the amino acid sequence of SEQ ID NO: 2 in Sequence Listing;

(B) a protein which has the amino acid sequence of SEQ ID NO: 2 in Sequence Listing including substitution, deletion, insertion, addition or inversion of one or several amino acids, and an activity for binding to sucrose.

3. The DNA according to Claim 2, which is a DNA defined in the following (a) or (b):

(a) a DNA which contains the nucleotide sequence of the nucleotides 3779 to 5761 of SEQ ID NO: 1 in Sequence Listing;

(b) a DNA which is hybridizable with a nucleotide sequence containing the nucleotide sequence of the

nucleotides 3779 to 5761 of SEQ ID NO: 1 in Sequence Listing under stringent conditions, and encodes a protein having an activity for binding to sucrose.

ABSTRACT

A gene encoding a protein constituting sucrose PTS of coryneform bacterium is provided by amplifying a region existing downstream from sucrase gene in coryneform bacterium by the cassette-ligation mediated PCR to obtain DNA encoding sucrose PTS enzyme II, which is a protein defined in the following (A) or (B):

(A) a protein which has the amino acid sequence of SEQ ID NO: 2 in Sequence Listing;

(B) a protein which has the amino acid sequence of SEQ ID NO: 2 in Sequence Listing including substitution, deletion, insertion, addition or inversion of one or several amino acids, and an activity for binding to sucrose.

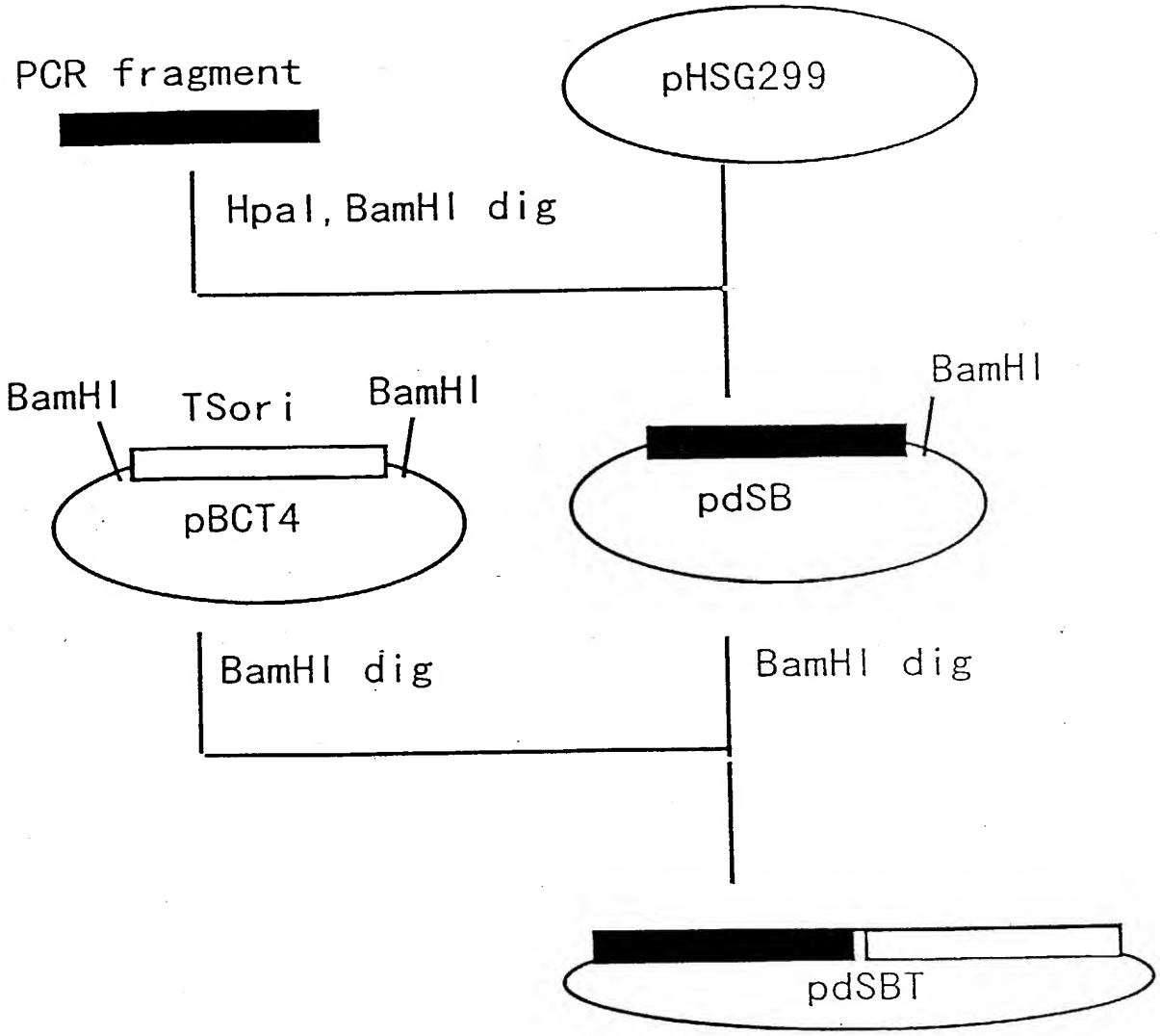
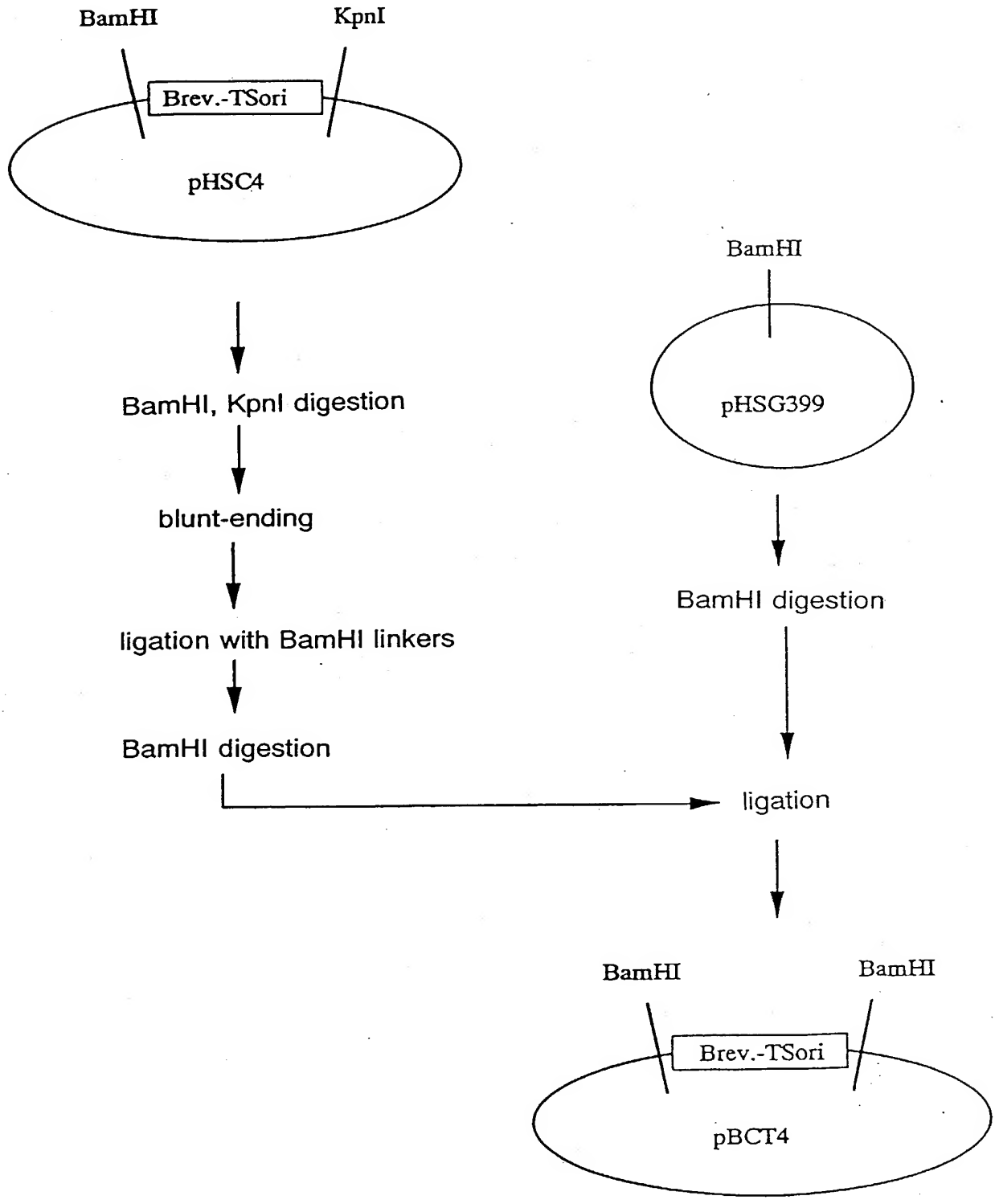


FIG.1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ligation with BamHI linkers
BamHI digestion
BamHI
pHSG399
BamHI digestion
ligation
BamHI
BamHI
Brev.-TSori
pBCT4

FIG.2

Declaration, Power Of Attorney and Petition

WE (I) the undersigned inventor(s), hereby declare(s) that:

My residence, post office address and citizenship are as stated below next to my name,

We (I) believe that we are (I am) the original, first, and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

DNA Encoding Sucrose PTS Enzyme II

the specification of which

- ☐ is attached hereto.
- ☐ was filed on _____ as
  Application Serial No. _____
  and amended on _____.
- ☒ was filed as PCT international application
  Number PCT/JP 00/04348
  on June 30, 2000,
  and was amended under PCT Article 19
  on _____ (if applicable).

We (I) hereby state that we (I) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We (I) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

We (I) hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed. Prior Foreign Application(s)

Application No.	Country	Day/Month/Year	Priority Claimed
11-189512	Japan	02/07/1999	☒ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No

We (I) hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application Number)	(Filing Date)
(Application Number)	(Filing Date)

We (I) hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.	Filing Date	Status (pending, patented, abandoned)

And we (I) hereby appoint: Norman F. Oblon, Registration Number 24,618; Marvin J. Spivak, Registration Number 24,913; C. Irvin McClelland, Registration Number 21,124; Gregory J. Maier, Registration Number 25,599; Arthur I. Neustadt, Registration Number 24,854; Richard D. Kelly, Registration Number 27,757; James D. Hamilton, Registration Number 28,421; Eckhard H. Kuesters, Registration Number 28,870; Robert T. Pous, Registration Number 29,099; Charles L. Gholz, Registration Number 26,395; Vincent J. Sunderdick, Registration Number 29,004; William E. Beaumont, Registration Number 30,996; Steven B. Kelber, Registration Number 30,073; Robert F. Gnuse, Registration Number 27,295; Jean-Paul Lavalleye, Registration Number 31,451; Timothy R. Schwartz, Registration Number 32,171; Stephen G. Baxter, Registration Number 32,884; Martin M. Zoltick, Registration Number 35,745; Robert W. Hahl, Registration Number 33,893; Richard L. Treanor, Registration Number 36,379; Steven P. Weihrouch, Registration Number 32,829; John T. Goolkasian, Registration Number 26,142; Marc R. Labgold, Registration Number 34,651; William J. Healey, Registration Number 36,160; and Richard L. Chinn, Registration Number 34,305; our (my) attorneys, with full powers of substitution and revocation, to prosecute this application and to transact all business in the Patent Office connected therewith; and we (I) hereby request that all correspondence regarding this application be sent to the firm of OBLON, SPIVAK, McCLELLAND, MAIER & NEUSTADT, P.C., whose Post Office Address is: Fourth Floor, 1755 Jefferson Davis Highway, Arlington, Virginia 22202.

We (I) declare that all statements made herein of our (my) own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Date

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Signature of Inventor

Date

SEQUENCE LISTING

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<130> B644MSOP1027

<150> JP 11-189512
<151> 1999-07-02

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ccacaaggat tggaccgaat ccacgatgag gttactcact acgcaagctc tgaccagtgc 3120
ggatatgtcg tcgacaagct tgaaggaacg accttccgcg tcttgcgagg attcagcgag 3180
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ctcgtgggct ggatggggct gcccgcgcag gatgatcacc caacagttgc acaggaagga 3300

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tgggtgcact gcctgactgt gccccgcaag cttcatttgc gcaaccacgc gatctaccaa 3360
gagctccttc tcccagaggg ggagtcgggg gtaatcagat ctgtattagg ttctgaacct 3420
gtccgagtag acatccgagg caatatttcc ctcgagtggg atggtgtccg tttgtctgtg 3480
gatcgtgatg gtgatcgtcg cgtagctgag gtaaaacctg gcgaattagt gatcgcggac 3540
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cttcaaaggt gacactattg agagataagt catataaaag ggtcttttgt ggcgaattgt 3660
acaaatactt cgcaaaatcc cttgatcgga cacaaataaa caggtttaat attgtttagc 3720
ttttgaacaa acattcatgt ctgaatattt ttgtttcttc ccggttaagg agaaattc   3778
atg gac cat aag gac ctc gcg caa cgc atc ctg cgc gac att ggc ggc   3826
Met Asp His Lys Asp Leu Ala Gln Arg Ile Leu Arg Asp Ile Gly Gly
 1               5                  10                  15
gaa gac aac att gtc gcc gcc gca cac tgt gca acg cgt tta cgc ctc   3874
Glu Asp Asn Ile Val Ala Ala Ala His Cys Ala Thr Arg Leu Arg Leu
            20                  25                  30
gtg ctc aaa gac acc aag gat gtg gat cgc caa agt ctg gat gat gat   3922
Val Leu Lys Asp Thr Lys Asp Val Asp Arg Gln Ser Leu Asp Asp Asp
        35                  40                  45
cca gat ctg aaa ggc acc ttt gaa act ggc ggc atg ttc cag atc atc   3970
Pro Asp Leu Lys Gly Thr Phe Glu Thr Gly Gly Met Phe Gln Ile Ile
    50                  55                  60
gtc ggg cca ggc gat gtg gat cat gtt ttc aaa gaa ctc gat gac gca   4018
Val Gly Pro Gly Asp Val Asp His Val Phe Lys Glu Leu Asp Asp Ala
65                  70                  75                  80
acc tcc aaa gac atc gct gtg tcc aca gag cag ctc aaa gat gtt gtg   4066
Thr Ser Lys Asp Ile Ala Val Ser Thr Glu Gln Leu Lys Asp Val Val
                85                  90                  95
gct aac aac gcc aac tgg ttc agc cgt gct gtg aag gta ttg gcg gac   4114
Ala Asn Asn Ala Asn Trp Phe Ser Arg Ala Val Lys Val Leu Ala Asp
            100                 105                 110
att ttc gtc ccg ctg att cca atc ttg gtt ggt ggc ggt ctg ctc atg   4162
Ile Phe Val Pro Leu Ile Pro Ile Leu Val Gly Gly Gly Leu Leu Met
        115                 120                 125
gct atc aac aat gtg ttg gtt gcg cag gat ctg ttc ggt ccg caa tca   4210
Ala Ile Asn Asn Val Leu Val Ala Gln Asp Leu Phe Gly Pro Gln Ser
    130                 135                 140
ctg gtg gag atg ttc cct cag atc agc ggt gtt gct gag atg atc aac   4258
Leu Val Glu Met Phe Pro Gln Ile Ser Gly Val Ala Glu Met Ile Asn
145                 150                 155                 160
ctg atg gca tct gcg ccg ttc gcg ttc ttg cca gtg ttg gtt ggt ttc   4306
Leu Met Ala Ser Ala Pro Phe Ala Phe Leu Pro Val Leu Val Gly Phe
                165                 170                 175
```

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acc gca acc aag cgt ttc ggt ggc aat gag ttc ctg ggc gcc ggc att   4354
Thr Ala Thr Lys Arg Phe Gly Gly Asn Glu Phe Leu Gly Ala Gly Ile
            180                 185                 190
ggt atg gcg atg gtg ttc cca acc ctg gtt aac ggc tac gac gtg gcc   4402
Gly Met Ala Met Val Phe Pro Thr Leu Val Asn Gly Tyr Asp Val Ala
        195                 200                 205
gcc acc atg acc gcg ggc gaa atg cca atg tgg tcc ctg ttt ggt ttg   4450
Ala Thr Met Thr Ala Gly Glu Met Pro Met Trp Ser Leu Phe Gly Leu
    210                 215                 220
gat gtt gct caa gct ggt tac cag ggc acc gtg ctt cct gtg ctg gtg   4498
Asp Val Ala Gln Ala Gly Tyr Gln Gly Thr Val Leu Pro Val Leu Val
225                 230                 235                 240
gtc tct tgg att ctg gca acg atc gag aag ttc ctg cac aag cga ctc   4546
Val Ser Trp Ile Leu Ala Thr Ile Glu Lys Phe Leu His Lys Arg Leu
                245                 250                 255
atg ggc act gca gac ttc ctg atc acc cca gtg ttg act ctg ctg ctc   4594
Met Gly Thr Ala Asp Phe Leu Ile Thr Pro Val Leu Thr Leu Leu Leu
            260                 265                 270
acc ggc ttc ctt acg ttc att gct att ggt cca gca atg cgc tgg gtg   4642
Thr Gly Phe Leu Thr Phe Ile Ala Ile Gly Pro Ala Met Arg Trp Val
        275                 280                 285
ggt gac ttg ctg gca cac ggt ctg cag gga ctc tat gat ttc ggt ggt   4690
Gly Asp Leu Leu Ala His Gly Leu Gln Gly Leu Tyr Asp Phe Gly Gly
    290                 295                 300
cca gtc ggc ggt ctg ctt ttc ggt ctg gtc tac tca cca atc gtt atc   4738
Pro Val Gly Gly Leu Leu Phe Gly Leu Val Tyr Ser Pro Ile Val Ile
305                 310                 315                 320
act ggt ctg cac cag tcc ttc ccg cca att gag ctg gag ctg ttc aac   4786
Thr Gly Leu His Gln Ser Phe Pro Pro Ile Glu Leu Glu Leu Phe Asn
                325                 330                 335
cag ggt gga tcc ttc atc ttc gca acc gca tcc atg gcc aat atc gcg   4834
Gln Gly Gly Ser Phe Ile Phe Ala Thr Ala Ser Met Ala Asn Ile Ala
            340                 345                 350
cag ggt gca gca tgt ttg gca gtg ttc ttc cta gcg aag agt gaa aag   4882
Gln Gly Ala Ala Cys Leu Ala Val Phe Phe Leu Ala Lys Ser Glu Lys
        355                 360                 365
ctc aag ggc ctt gca ggt gct tca ggt gtc tcc gct gtt ctt ggt att   4930
Leu Lys Gly Leu Ala Gly Ala Ser Gly Val Ser Ala Val Leu Gly Ile
    370                 375                 380
aca gag cct gcg atc ttc ggt gtg aac ctt cgc ctg cgc tgg ccg ttc   4978
Thr Glu Pro Ala Ile Phe Gly Val Asn Leu Arg Leu Arg Trp Pro Phe
```

```
385                 390                 395                 400
tac att ggt atc ggt acc gca gct atc ggt ggc gct ttg att gca ctc   5026
Tyr Ile Gly Ile Gly Thr Ala Ala Ile Gly Gly Ala Leu Ile Ala Leu
                405                 410                 415
ttt gat atc aag gca gtt gcg ttg ggc gct gca ggt ttc ttg ggt gtt   5074
Phe Asp Ile Lys Ala Val Ala Leu Gly Ala Ala Gly Phe Leu Gly Val
            420                 425                 430
gtt tct att gat gct cca gat atg gtc atg ttc ttg gtt tgc gcg gta   5122
Val Ser Ile Asp Ala Pro Asp Met Val Met Phe Leu Val Cys Ala Val
        435                 440                 445
gtt acc ttt gtc atc gca ttc ggc gca gcg att gct tat ggc ctt tac   5170
Val Thr Phe Val Ile Ala Phe Gly Ala Ala Ile Ala Tyr Gly Leu Tyr
    450                 455                 460
ttg gtt cgc cgc aac ggc agc att gat cca gat gca acc gct gct cca   5218
Leu Val Arg Arg Asn Gly Ser Ile Asp Pro Asp Ala Thr Ala Ala Pro
465                 470                 475                 480
gtg cct gca gga acg acc aaa gcc gaa gca gaa gca ccc gca gaa ttt   5266
Val Pro Ala Gly Thr Thr Lys Ala Glu Ala Glu Ala Pro Ala Glu Phe
                485                 490                 495
tca aac gat tcc acc atc atc cag gca cct ttg acc ggt gaa gct atc   5314
Ser Asn Asp Ser Thr Ile Ile Gln Ala Pro Leu Thr Gly Glu Ala Ile
            500                 505                 510
gca ctg agc agc gtc agc gat gcc atg ttt gcc agc gga aag ctt ggc   5362
Ala Leu Ser Ser Val Ser Asp Ala Met Phe Ala Ser Gly Lys Leu Gly
        515                 520                 525
tca ggt gtt gcg atc gtc ccc acc aag ggg cag ctg gtt tca cca gtg   5410
Ser Gly Val Ala Ile Val Pro Thr Lys Gly Gln Leu Val Ser Pro Val
    530                 535                 540
agc gga aag atc gtg gtg gcc ttc cca tct ggt cac gct ttc gca gtc   5458
Ser Gly Lys Ile Val Val Ala Phe Pro Ser Gly His Ala Phe Ala Val
545                 550                 555                 560
cgc act aag gct gag gat ggt tcc aat gtg gat atc ttg atg cac att   5506
Arg Thr Lys Ala Glu Asp Gly Ser Asn Val Asp Ile Leu Met His Ile
                565                 570                 575
ggt ttc gac acc gta aac ctc aac ggc acg cac ttt aac ccg ctg aag   5554
Gly Phe Asp Thr Val Asn Leu Asn Gly Thr His Phe Asn Pro Leu Lys
            580                 585                 590
aag cag ggc gat gaa gtc aaa gca ggg gag ctg ctg tgt gaa ttc gat   5602
Lys Gln Gly Asp Glu Val Lys Ala Gly Glu Leu Leu Cys Glu Phe Asp
        595                 600                 605
att gat gcc att aag gct gca ggt tat gag gta acc acg ccg att gtt   5650
```

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Ile Asp Ala Ile Lys Ala Ala Gly Tyr Glu Val Thr Thr Pro Ile Val
    610                 615                 620
gtt tcg aat tac aag aaa acc gga cct gta aac act tac ggt ttg ggc   5698
Val Ser Asn Tyr Lys Lys Thr Gly Pro Val Asn Thr Tyr Gly Leu Gly
625                 630                 635                 640
gaa att gaa gcg gga gcc aac ctg ctc aac gtc gca aag aaa gaa gcg   5746
Glu Ile Glu Ala Gly Ala Asn Leu Leu Asn Val Ala Lys Lys Glu Ala
                645                 650                 655
gtg cca gca aca cca taagttgaaa ccttgagtgt tcgcacacag gttagactag   5801
Val Pro Ala Thr Pro
            660
gggacgtgac tctacgcatc tttgacaccg gtacccgtac gcttcgagat tttaaacctg 5861
ttcaaccagg tcatgcctcg gtgtacctgt gtggtgccac cccgcaatct tcaccccaca 5921
ttggacatgt tcgttcagca gtagcgtttg atattttgcg ccgctgaa              5969

<210> 2
<211> 661
<212> PRT
<213> Brevibacterium lactofermentum

<400> 2
Met Asp His Lys Asp Leu Ala Gln Arg Ile Leu Arg Asp Ile Gly Gly
  1               5                  10                  15
Glu Asp Asn Ile Val Ala Ala Ala His Cys Ala Thr Arg Leu Arg Leu
             20                  25                  30
Val Leu Lys Asp Thr Lys Asp Val Asp Arg Gln Ser Leu Asp Asp Asp
         35                  40                  45
Pro Asp Leu Lys Gly Thr Phe Glu Thr Gly Gly Met Phe Gln Ile Ile
     50                  55                  60
Val Gly Pro Gly Asp Val Asp His Val Phe Lys Glu Leu Asp Asp Ala
 65                  70                  75                  80
Thr Ser Lys Asp Ile Ala Val Ser Thr Glu Gln Leu Lys Asp Val Val
                 85                  90                  95
Ala Asn Asn Ala Asn Trp Phe Ser Arg Ala Val Lys Val Leu Ala Asp
            100                 105                 110
Ile Phe Val Pro Leu Ile Pro Ile Leu Val Gly Gly Gly Leu Leu Met
        115                 120                 125
Ala Ile Asn Asn Val Leu Val Ala Gln Asp Leu Phe Gly Pro Gln Ser
    130                 135                 140
Leu Val Glu Met Phe Pro Gln Ile Ser Gly Val Ala Glu Met Ile Asn
145                 150                 155                 160
```

Leu Met Ala Ser Ala Pro Phe Ala Phe Leu Pro Val Leu Val Gly Phe
165 170 175
Thr Ala Thr Lys Arg Phe Gly Gly Asn Glu Phe Leu Gly Ala Gly Ile
180 185 190
Gly Met Ala Met Val Phe Pro Thr Leu Val Asn Gly Tyr Asp Val Ala
195 200 205
Ala Thr Met Thr Ala Gly Glu Met Pro Met Trp Ser Leu Phe Gly Leu
210 215 220
Asp Val Ala Gln Ala Gly Tyr Gln Gly Thr Val Leu Pro Val Leu Val
225 230 235 240
Val Ser Trp Ile Leu Ala Thr Ile Glu Lys Phe Leu His Lys Arg Leu
245 250 255
Met Gly Thr Ala Asp Phe Leu Ile Thr Pro Val Leu Thr Leu Leu Leu
260 265 270
Thr Gly Phe Leu Thr Phe Ile Ala Ile Gly Pro Ala Met Arg Trp Val
275 280 285
Gly Asp Leu Leu Ala His Gly Leu Gln Gly Leu Tyr Asp Phe Gly Gly
290 295 300
Pro Val Gly Gly Leu Leu Phe Gly Leu Val Tyr Ser Pro Ile Val Ile
305 310 315 320
Thr Gly Leu His Gln Ser Phe Pro Pro Ile Glu Leu Glu Leu Phe Asn
325 330 335
Gln Gly Gly Ser Phe Ile Phe Ala Thr Ala Ser Met Ala Asn Ile Ala
340 345 350
Gln Gly Ala Ala Cys Leu Ala Val Phe Phe Leu Ala Lys Ser Glu Lys
355 360 365
Leu Lys Gly Leu Ala Gly Ala Ser Gly Val Ser Ala Val Leu Gly Ile
370 375 380
Thr Glu Pro Ala Ile Phe Gly Val Asn Leu Arg Leu Arg Trp Pro Phe
385 390 395 400
Tyr Ile Gly Ile Gly Thr Ala Ala Ile Gly Gly Ala Leu Ile Ala Leu
405 410 415
Phe Asp Ile Lys Ala Val Ala Leu Gly Ala Ala Gly Phe Leu Gly Val
420 425 430
Val Ser Ile Asp Ala Pro Asp Met Val Met Phe Leu Val Cys Ala Val
435 440 445
Val Thr Phe Val Ile Ala Phe Gly Ala Ala Ile Ala Tyr Gly Leu Tyr
450 455 460
Leu Val Arg Arg Asn Gly Ser Ile Asp Pro Asp Ala Thr Ala Ala Pro
465 470 475 480
Val Pro Ala Gly Thr Thr Lys Ala Glu Ala Glu Ala Pro Ala Glu Phe

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                485                 490                 495
Ser Asn Asp Ser Thr Ile Ile Gln Ala Pro Leu Thr Gly Glu Ala Ile
            500                 505                 510
Ala Leu Ser Ser Val Ser Asp Ala Met Phe Ala Ser Gly Lys Leu Gly
        515                 520                 525
Ser Gly Val Ala Ile Val Pro Thr Lys Gly Gln Leu Val Ser Pro Val
    530                 535                 540
Ser Gly Lys Ile Val Val Ala Phe Pro Ser Gly His Ala Phe Ala Val
545                 550                 555                 560
Arg Thr Lys Ala Glu Asp Gly Ser Asn Val Asp Ile Leu Met His Ile
                565                 570                 575
Gly Phe Asp Thr Val Asn Leu Asn Gly Thr His Phe Asn Pro Leu Lys
            580                 585                 590
Lys Gln Gly Asp Glu Val Lys Ala Gly Glu Leu Leu Cys Glu Phe Asp
        595                 600                 605
Ile Asp Ala Ile Lys Ala Ala Gly Tyr Glu Val Thr Thr Pro Ile Val
    610                 615                 620
Val Ser Asn Tyr Lys Lys Thr Gly Pro Val Asn Thr Tyr Gly Leu Gly
625                 630                 635                 640
Glu Ile Glu Ala Gly Ala Asn Leu Leu Asn Val Ala Lys Lys Glu Ala
                645                 650                 655
Val Pro Ala Thr Pro
            660
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<210> 3
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Sau3AI cassette

<220>
<221> misc_feature
<222> (44)
<223> complementary strand extends a single strand having
a sequence of 3'-ctag-5' at this position in the
direction of 5' from 3'

<400> 3
gtacatattg tcgttagaac gcgtaatacg actcactata ggga 44

<210> 4
<211> 47
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: EcoRI cassette

<220>
<221> misc_feature
<222> (47)
<223> complementary strand extends a single strand having
a sequence of 3'-ttaa-5' at this position in the
direction of 5' from 3'

<400> 4
gtacatattg tcgttagaac gcgtaatacg actcactata gggagag 47

<210> 5
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: HindIII cassette

<220>
<221> misc_feature
<222> (46)
<223> complementary strand extends a single strand having
a sequence of 3'-tcga-5' at this position in the
direction of 5' from 3'

<400> 5
gtacatattg tcgttagaac gcgtaatacg actcactata gggaga 46

<210> 6
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PstI cassette
<220>
<221> misc_feature
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<223> complementary strand does not exist

<400> 6
gtacatattg tcgttagaac gcgtaatacg actcactata gggagactgc a 51

<210> 7
<211> 47
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: SalI cassette

<220>
<221> misc_feature
<222> (47)
<223> complementary strand extends a single strand having
a sequence of 3'-agct-5' at this position in the
direction of 5' from 3'

<400> 7
gtacatattg tcgttagaac gcgtaatacg actcactata gggagag 47

<210> 8
<211> 47
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: XbaI cassette

<220>
<221> misc_feature
<222> (47)
<223> complementary strand extends a single strand having

a sequence of 3'-gatc-5' at this position in the direction of 5' from 3'

<400> 8
gtacatattg tcgttagaac gcgtaatacg actcactata gggagat 47

<210> 9
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for PCR

<400> 9
cgtcttgcga ggattcagcg agctg 25

<210> 10
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for PCR

<400> 10
agctggattt cggccatgaa ttcta 25

<210> 11
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for PCR

<400> 11
gatctgttcg gtccgcaatc act 23

<210> 12
<211> 25

<212> DNA
<213> Artificial Sequence

<220>
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<400> 12
cactggtgga gatgttccct cagat 25

<210> 13
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for PCR

<400> 13
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<210> 14
<211> 24
<212> DNA
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<220>
<223> Description of Artificial Sequence: primer for PCR
<400> 14
cgcgcagggt gcagcatgtt tggc 24

<210> 15
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for PCR

<400> 15
gggccttgca ggtgcttcag gtgtc 25

<210> 16
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for PCR

<400> 16
ccgctgttct tggtattaca gagcc 25

<210> 17
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for PCR

<400> 17
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<210> 18
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for PCR

<400> 18
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<210> 19
<211> 36
<212> DNA
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<220>
<223> Description of Artificial Sequence: cassette
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<400> 19
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<210> 20
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: cassette primer 2

<400> 20
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<210> 21
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for PCR

<400> 21
cgctactgct gaacgaacat gtcc 24

SEQUENCE LISTING

<110> IZUI, MASAKO
SUGIMOTO, MASAKAZU
KURAHASHI, OSAMU
NAKAMATSU, TSUYOSHI

<120> DNA ENCODING SUCROSE PTS ENZYME II

<130> 217677US0PCT

<140> US 10/019,284
<141> 2002-01-02

<150> JP 11-189512
<151> 1999-07-02

<160> 21

<170> PatentIn version 3.1

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gaacccatgg tgttccttgt gggttgaggg aacgagtgcg ggtgagaagt ttttcaagtg 180

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caacagcagg cctcaagtcc gaagataatt aacctagatc cgtagacata agacatcata 300

cgtcctatgc ttgctggaag gaaccaaata acctcagaaa gatggcagaa gtggtgcatt 360

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ttttgaacaa acattcatgt ctgaatattt ttgtttcttc ccggttaagg agaaattc 3778

atg gac cat aag gac ctc gcg caa cgc atc ctg cgc gac att ggc ggc 3826
Met Asp His Lys Asp Leu Ala Gln Arg Ile Leu Arg Asp Ile Gly Gly
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gaa gac aac att gtc gcc gcc gca cac tgt gca acg cgt tta cgc ctc 3874
Glu Asp Asn Ile Val Ala Ala Ala His Cys Ala Thr Arg Leu Arg Leu
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gtg ctc aaa gac acc aag gat gtg gat cgc caa agt ctg gat gat gat 3922
Val Leu Lys Asp Thr Lys Asp Val Asp Arg Gln Ser Leu Asp Asp Asp
35 40 45

cca gat ctg aaa ggc acc ttt gaa act ggc ggc atg ttc cag atc atc 3970

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Pro Asp Leu Lys Gly Thr Phe Glu Thr Gly Gly Met Phe Gln Ile Ile
    50                  55                  60

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Val Gly Pro Gly Asp Val Asp His Val Phe Lys Glu Leu Asp Asp Ala
65                  70                  75                  80

acc tcc aaa gac atc gct gtg tcc aca gag cag ctc aaa gat gtt gtg      4066
Thr Ser Lys Asp Ile Ala Val Ser Thr Glu Gln Leu Lys Asp Val Val
                85                  90                  95

gct aac aac gcc aac tgg ttc agc cgt gct gtg aag gta ttg gcg gac      4114
Ala Asn Asn Ala Asn Trp Phe Ser Arg Ala Val Lys Val Leu Ala Asp
            100                 105                 110

att ttc gtc ccg ctg att cca atc ttg gtt ggt ggc ggt ctg ctc atg      4162
Ile Phe Val Pro Leu Ile Pro Ile Leu Val Gly Gly Gly Leu Leu Met
        115                 120                 125

gct atc aac aat gtg ttg gtt gcg cag gat ctg ttc ggt ccg caa tca      4210
Ala Ile Asn Asn Val Leu Val Ala Gln Asp Leu Phe Gly Pro Gln Ser
    130                 135                 140

ctg gtg gag atg ttc cct cag atc agc ggt gtt gct gag atg atc aac      4258
Leu Val Glu Met Phe Pro Gln Ile Ser Gly Val Ala Glu Met Ile Asn
145                 150                 155                 160

ctg atg gca tct gcg ccg ttc gcg ttc ttg cca gtg ttg gtt ggt ttc      4306
Leu Met Ala Ser Ala Pro Phe Ala Phe Leu Pro Val Leu Val Gly Phe
                165                 170                 175

acc gca acc aag cgt ttc ggt ggc aat gag ttc ctg ggc gcc ggc att      4354
Thr Ala Thr Lys Arg Phe Gly Gly Asn Glu Phe Leu Gly Ala Gly Ile
            180                 185                 190

ggt atg gcg atg gtg ttc cca acc ctg gtt aac ggc tac gac gtg gcc      4402
Gly Met Ala Met Val Phe Pro Thr Leu Val Asn Gly Tyr Asp Val Ala
        195                 200                 205

gcc acc atg acc gcg ggc gaa atg cca atg tgg tcc ctg ttt ggt ttg      4450
Ala Thr Met Thr Ala Gly Glu Met Pro Met Trp Ser Leu Phe Gly Leu
    210                 215                 220

gat gtt gct caa gct ggt tac cag ggc acc gtg ctt cct gtg ctg gtg      4498
Asp Val Ala Gln Ala Gly Tyr Gln Gly Thr Val Leu Pro Val Leu Val
225                 230                 235                 240

gtc tct tgg att ctg gca acg atc gag aag ttc ctg cac aag cga ctc      4546
Val Ser Trp Ile Leu Ala Thr Ile Glu Lys Phe Leu His Lys Arg Leu
                245                 250                 255

atg ggc act gca gac ttc ctg atc acc cca gtg ttg act ctg ctg ctc      4594
Met Gly Thr Ala Asp Phe Leu Ile Thr Pro Val Leu Thr Leu Leu Leu
            260                 265                 270

acc ggc ttc ctt acg ttc att gct att ggt cca gca atg cgc tgg gtg      4642
Thr Gly Phe Leu Thr Phe Ile Ala Ile Gly Pro Ala Met Arg Trp Val
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ggt gac ttg ctg gca cac ggt ctg cag gga ctc tat gat ttc ggt ggt     4690
Gly Asp Leu Leu Ala His Gly Leu Gln Gly Leu Tyr Asp Phe Gly Gly
    290                 295                 300

cca gtc ggc ggt ctg ctt ttc ggt ctg gtc tac tca cca atc gtt atc     4738
Pro Val Gly Gly Leu Leu Phe Gly Leu Val Tyr Ser Pro Ile Val Ile
305                 310                 315                 320

act ggt ctg cac cag tcc ttc ccg cca att gag ctg gag ctg ttc aac     4786
Thr Gly Leu His Gln Ser Phe Pro Pro Ile Glu Leu Glu Leu Phe Asn
                325                 330                 335

cag ggt gga tcc ttc atc ttc gca acc gca tcc atg gcc aat atc gcg     4834
Gln Gly Gly Ser Phe Ile Phe Ala Thr Ala Ser Met Ala Asn Ile Ala
            340                 345                 350

cag ggt gca gca tgt ttg gca gtg ttc ttc cta gcg aag agt gaa aag     4882
Gln Gly Ala Ala Cys Leu Ala Val Phe Phe Leu Ala Lys Ser Glu Lys
        355                 360                 365

ctc aag ggc ctt gca ggt gct tca ggt gtc tcc gct gtt ctt ggt att     4930
Leu Lys Gly Leu Ala Gly Ala Ser Gly Val Ser Ala Val Leu Gly Ile
    370                 375                 380

aca gag cct gcg atc ttc ggt gtg aac ctt cgc ctg cgc tgg ccg ttc     4978
Thr Glu Pro Ala Ile Phe Gly Val Asn Leu Arg Leu Arg Trp Pro Phe
385                 390                 395                 400

tac att ggt atc ggt acc gca gct atc ggt ggc gct ttg att gca ctc     5026
Tyr Ile Gly Ile Gly Thr Ala Ala Ile Gly Gly Ala Leu Ile Ala Leu
                405                 410                 415

ttt gat atc aag gca gtt gcg ttg ggc gct gca ggt ttc ttg ggt gtt     5074
Phe Asp Ile Lys Ala Val Ala Leu Gly Ala Ala Gly Phe Leu Gly Val
            420                 425                 430

gtt tct att gat gct cca gat atg gtc atg ttc ttg gtt tgc gcg gta     5122
Val Ser Ile Asp Ala Pro Asp Met Val Met Phe Leu Val Cys Ala Val
        435                 440                 445

gtt acc ttt gtc atc gca ttc ggc gca gcg att gct tat ggc ctt tac     5170
Val Thr Phe Val Ile Ala Phe Gly Ala Ala Ile Ala Tyr Gly Leu Tyr
    450                 455                 460

ttg gtt cgc cgc aac ggc agc att gat cca gat gca acc gct gct cca     5218
Leu Val Arg Arg Asn Gly Ser Ile Asp Pro Asp Ala Thr Ala Ala Pro
465                 470                 475                 480

gtg cct gca gga acg acc aaa gcc gaa gca gaa gca ccc gca gaa ttt     5266
Val Pro Ala Gly Thr Thr Lys Ala Glu Ala Glu Ala Pro Ala Glu Phe
                485                 490                 495

tca aac gat tcc acc atc atc cag gca cct ttg acc ggt gaa gct atc     5314
Ser Asn Asp Ser Thr Ile Ile Gln Ala Pro Leu Thr Gly Glu Ala Ile
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Ala Leu Ser Ser Val Ser Asp Ala Met Phe Ala Ser Gly Lys Leu Gly
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tca ggt gtt gcg atc gtc ccc acc aag ggg cag ctg gtt tca cca gtg      5410
Ser Gly Val Ala Ile Val Pro Thr Lys Gly Gln Leu Val Ser Pro Val
    530                 535                 540

agc gga aag atc gtg gtg gcc ttc cca tct ggt cac gct ttc gca gtc      5458
Ser Gly Lys Ile Val Val Ala Phe Pro Ser Gly His Ala Phe Ala Val
545                 550                 555                 560

cgc act aag gct gag gat ggt tcc aat gtg gat atc ttg atg cac att      5506
Arg Thr Lys Ala Glu Asp Gly Ser Asn Val Asp Ile Leu Met His Ile
                565                 570                 575

ggt ttc gac acc gta aac ctc aac ggc acg cac ttt aac ccg ctg aag      5554
Gly Phe Asp Thr Val Asn Leu Asn Gly Thr His Phe Asn Pro Leu Lys
            580                 585                 590

aag cag ggc gat gaa gtc aaa gca ggg gag ctg ctg tgt gaa ttc gat      5602
Lys Gln Gly Asp Glu Val Lys Ala Gly Glu Leu Leu Cys Glu Phe Asp
        595                 600                 605

att gat gcc att aag gct gca ggt tat gag gta acc acg ccg att gtt      5650
Ile Asp Ala Ile Lys Ala Ala Gly Tyr Glu Val Thr Thr Pro Ile Val
    610                 615                 620

gtt tcg aat tac aag aaa acc gga cct gta aac act tac ggt ttg ggc      5698
Val Ser Asn Tyr Lys Lys Thr Gly Pro Val Asn Thr Tyr Gly Leu Gly
625                 630                 635                 640

gaa att gaa gcg gga gcc aac ctg ctc aac gtc gca aag aaa gaa gcg      5746
Glu Ile Glu Ala Gly Ala Asn Leu Leu Asn Val Ala Lys Lys Glu Ala
                645                 650                 655

gtg cca gca aca cca taagttgaaa ccttgagtgt tcgcacacag gttagactag      5801
Val Pro Ala Thr Pro
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gggacgtgac tctacgcatc tttgacaccg gtacccgtac gcttcgagat tttaaacctg    5861

ttcaaccagg tcatgcctcg gtgtacctgt gtggtgccac cccgcaatct tcaccccaca    5921

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Val Leu Lys Asp Thr Lys Asp Val Asp Arg Gln Ser Leu Asp Asp Asp
        35                  40                  45

Pro Asp Leu Lys Gly Thr Phe Glu Thr Gly Gly Met Phe Gln Ile Ile
    50                  55                  60

Val Gly Pro Gly Asp Val Asp His Val Phe Lys Glu Leu Asp Asp Ala
65                  70                  75                  80

Thr Ser Lys Asp Ile Ala Val Ser Thr Glu Gln Leu Lys Asp Val Val
                85                  90                  95

Ala Asn Asn Ala Asn Trp Phe Ser Arg Ala Val Lys Val Leu Ala Asp
            100                 105                 110

Ile Phe Val Pro Leu Ile Pro Ile Leu Val Gly Gly Gly Leu Leu Met
        115                 120                 125

Ala Ile Asn Asn Val Leu Val Ala Gln Asp Leu Phe Gly Pro Gln Ser
    130                 135                 140

Leu Val Glu Met Phe Pro Gln Ile Ser Gly Val Ala Glu Met Ile Asn
145                 150                 155                 160

Leu Met Ala Ser Ala Pro Phe Ala Phe Leu Pro Val Leu Val Gly Phe
                165                 170                 175

Thr Ala Thr Lys Arg Phe Gly Gly Asn Glu Phe Leu Gly Ala Gly Ile
            180                 185                 190

Gly Met Ala Met Val Phe Pro Thr Leu Val Asn Gly Tyr Asp Val Ala
        195                 200                 205

Ala Thr Met Thr Ala Gly Glu Met Pro Met Trp Ser Leu Phe Gly Leu
    210                 215                 220

Asp Val Ala Gln Ala Gly Tyr Gln Gly Thr Val Leu Pro Val Leu Val
225                 230                 235                 240
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Val Ser Trp Ile Leu Ala Thr Ile Glu Lys Phe Leu His Lys Arg Leu
                245                 250                 255

Met Gly Thr Ala Asp Phe Leu Ile Thr Pro Val Leu Thr Leu Leu Leu
            260                 265                 270

Thr Gly Phe Leu Thr Phe Ile Ala Ile Gly Pro Ala Met Arg Trp Val
        275                 280                 285

Gly Asp Leu Leu Ala His Gly Leu Gln Gly Leu Tyr Asp Phe Gly Gly
    290                 295                 300

Pro Val Gly Gly Leu Leu Phe Gly Leu Val Tyr Ser Pro Ile Val Ile
305                 310                 315                 320

Thr Gly Leu His Gln Ser Phe Pro Pro Ile Glu Leu Glu Leu Phe Asn
                325                 330                 335

Gln Gly Gly Ser Phe Ile Phe Ala Thr Ala Ser Met Ala Asn Ile Ala
            340                 345                 350

Gln Gly Ala Ala Cys Leu Ala Val Phe Phe Leu Ala Lys Ser Glu Lys
        355                 360                 365

Leu Lys Gly Leu Ala Gly Ala Ser Gly Val Ser Ala Val Leu Gly Ile
    370                 375                 380

Thr Glu Pro Ala Ile Phe Gly Val Asn Leu Arg Leu Arg Trp Pro Phe
385                 390                 395                 400

Tyr Ile Gly Ile Gly Thr Ala Ala Ile Gly Gly Ala Leu Ile Ala Leu
                405                 410                 415

Phe Asp Ile Lys Ala Val Ala Leu Gly Ala Ala Gly Phe Leu Gly Val
            420                 425                 430

Val Ser Ile Asp Ala Pro Asp Met Val Met Phe Leu Val Cys Ala Val
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Val Thr Phe Val Ile Ala Phe Gly Ala Ala Ile Ala Tyr Gly Leu Tyr
    450                 455                 460
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Leu Val Arg Arg Asn Gly Ser Ile Asp Pro Asp Ala Thr Ala Ala Pro
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Val Pro Ala Gly Thr Thr Lys Ala Glu Ala Glu Ala Pro Ala Glu Phe
485 490 495

Ser Asn Asp Ser Thr Ile Ile Gln Ala Pro Leu Thr Gly Glu Ala Ile
500 505 510

Ala Leu Ser Ser Val Ser Asp Ala Met Phe Ala Ser Gly Lys Leu Gly
515 520 525

Ser Gly Val Ala Ile Val Pro Thr Lys Gly Gln Leu Val Ser Pro Val
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Ser Gly Lys Ile Val Val Ala Phe Pro Ser Gly His Ala Phe Ala Val
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Arg Thr Lys Ala Glu Asp Gly Ser Asn Val Asp Ile Leu Met His Ile
565 570 575

Gly Phe Asp Thr Val Asn Leu Asn Gly Thr His Phe Asn Pro Leu Lys
580 585 590

Lys Gln Gly Asp Glu Val Lys Ala Gly Glu Leu Leu Cys Glu Phe Asp
595 600 605

Ile Asp Ala Ile Lys Ala Ala Gly Tyr Glu Val Thr Thr Pro Ile Val
610 615 620

Val Ser Asn Tyr Lys Lys Thr Gly Pro Val Asn Thr Tyr Gly Leu Gly
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